CHEMIJA. 2018. Vol. 29. No. 2. P. 127–134

Cytotoxic attributes of antiviral plants rich in polyphenolic and volatile compounds

Tomas Drevinskas[1],

Rūta Mickienė[1],

Audrius Maruška[1*],

Mantas Stankevičius[1],

Nicola Tiso[1],

Algirdas Šalomskas[2],

Raimundas Lelešius[3, 2],

Agneta Karpovaitė[2],

Ona Ragažinskienė[4]

[1] *Faculty of Natural Sciences,*
Instrumental Analysis Open Access Centre,
Vytautas Magnus University,
Vileikos St. 8, 44404 Kaunas, Lithuania

[2] *Department of Veterinary Pathobiology,*
Veterinary Academy of Lithuanian University
of Health Science,
Tilžės St. 18, 47181 Kaunas, Lithuania

[3] *Institute of Microbiology and Virology,*
Veterinary Academy of Lithuanian University
of Health Science,
Tilžės St. 18, 47181 Kaunas, Lithuania

[4] *Sector of Medicinal Plants,*
Kaunas Botanical Garden
of Vytautas Magnus University,
Ž. E. Žilibero St. 6,
46324 Kaunas, Lithuania

The application of natural products for treatment and prevention of various diseases is becoming more important. Not only antibacterial natural preparations are being developed, but also antiviral natural products are investigated. There are two main problems in such investigations: (i) special chemical analysis and data analysis methods have to be used, (ii) not only antiviral effect has to be taken into account, but also cytotoxic and other unwanted effects. In this work, a combination of separation methods (capillary electrophoresis and gas chromatography), spectrophotometric methods, data mining/data analysis methods (hierarchical clusterization, multidimensional scaling and classification and regression tree induction) for clarifying chemical attributes in cytotoxic plant extracts was applied. The selected plants belonged to families: (i) Lamiaceae, (ii) Asteraceae and (iii) Fabaceae. Most cytotoxic were *Agastache foeniculum* Pursh. Kuntze. and *Salvia officinalis* L. extracts. Least cytotoxic were *Perilla frutescens* L. and *Satureja Montana* L. extracts. The tendency of cytotoxic extracts having abundant concentrations of medium and low electrophoretic mobility cations was clarified.

Keywords: chemometrics, capillary electrophoresis, gas chromatography, spectrophotometrics, antiviral plants, data mining

* Corresponding author. Email: audrius.maruska@vdu.lt

INTRODUCTION

Medicinal plants and products derived from medicinal plants are top priority among phytotherapy scientists [1]. Such products are interesting due to the fact that they are from the source of natural origin [1]. Bactericidal and bacteriostatic effects of plant extracts are widely investigated and known [2]. Antiviral effects are also reported in the literature [3]. Despite all these beneficial effects of the medicinal plants, it must be taken into account that plant extracts possess negative effects: (i) toxic, (ii) mutagenic, (iii) carcinogenic, (iv) hallucinogenic, (v) allergic and other unwanted effects [4]. It is known in the literature that numerous lethal and potentially lethal plants exist [5]. Utmost care must be taken developing pharmaceutical preparations due to the fact that residual toxic substances left in the developed products can lead to serious consequences [4].

Separation methods are superior over standard electrochemical, or spectrophotometric methods, since the separation step is combined with spectrometric, electrochemical or other physicochemical detection methods. Separation methods can clarify multiple factors, or peaks in a single run, therefore such means can be treated as multidimensional information providing methods [6]. Among them capillary electrophoresis, gas chromatography and liquid chromatography are dominating in phytochemical investigations [7, 8]. Capillary electrophoresis is a method that is used for separation of soluble charged, or chargeable analytes [9]. Gas chromatography is mainly used for separation of volatile compounds [10]. Liquid chromatography in the reversed phase mode is mainly used for separating substances according to their different pollarity [11]. Spectrophotometric assays are also used. Such methods are capable of determining certain groups of compounds in the complex mixtures and such techniques help in screening the substances of interest [10, 11].

Multiple data analysis methods exist for clarifying complex tendencies. Currently, machine learning and data mining methods are gaining popularity due to the fact that computational power of the computers has increased significantly since such methods were invented [12]. Deep neural networks and their modifications are used in computational biology [13]. Decision tree methods are capable of classifying various cases and even bio-products [14]. The k-Nearest-Neighbor classification has been used in the past and is still widely used now for biological and biochemical investigations [15]. In some cases subjective features have to be evaluated and expressed numerically and such approach has already been applied for evaluation of kiwifruit natural coatings [16]. Recently a methodology for determination of antiviral attributes in the plant extracts has been published [17]. It was determined that plant extracts contained phenolic compounds that were related to antiviral activity in the plant extracts. Additionally, amine group containing substances were separated suggesting that they are indirectly related to antiviral activity in medicinal plant extracts [17].

The aim of this work was to clarify what attributes in potentially antiviral plants possess cytotoxic activity.

EXPERIMENTAL

Chemicals

Acetic acid was purchased from Reachem (Slovakia). Methanol (MeOH) (99.9%), acetonitrile (ACN) (99.9%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalceau reagent 2N and rutin (95%) and MTT reagent were purchased from Sigma-Aldrich (Germany), dimethyl sulfoxide (DMSO) was from Carl Roth (Germany). Aluminum chloride was from ROTH (USA). Sodium hydroxide (99.0%) was purchased from Reachem (Slovakia), ethanol (EtOH) (96%) was from Sigma-Aldrich (Germany). Bidistilled water was produced in the laboratory using a Fistreem Cyclon bidistillator (UK). Detector helium gas (detector grade) for gas chromatography was from AGA, Latvia.

Instrumentation

The capillary electrophoresis (CE) system HP3DCE was used for analysis of plant extracts. The CE system had a contactless conductivity detector integrated following the previously published procedures [18, 19]. The gas chromatography–mass spectrometry system GC2010 and GCMS-QP2010 (Shimadzu, Japan) was used for the analysis of volatiles. The samples were handled using an automatic injector AOC-5000 from Shimadzu (Japan). The capillary column RTX-5MS, length 30 m, ID 0.25 mm, film thickness 0.25 μm (Restek, USA), was used for the separation of volatile compounds.

The Milton Roy Spectronic 1201 Spectrophotometer was used for photometric assays.

Sample preparation

Medicinal plants rich in volatile and polyphenol compounds were selected for the research [20]. The set of selected plants was previously investigated for antiviral effects [17]. The list of plants is the following: *Saturea montana* L. (*S. montana*), *Chamaemelum nobile* L. (*Ch. nobile*), *Perilla frutescens* L. Britton. (*P. frutescens*), *Agastache foeniculum* (Pursh) Kuntze (*A. foeniculum)*, *Origanum vulgare* L. (*O. vulgare*), *Mentha piperita* L. (*M. piperita)*, *Geranium macrorrhizum* L. (*G. macrorrhizum*), *Melissa officinalis* L. (*M. officinalis*), *Angelica archangelica* L. (aerial part) (*A. archangelica* aerial part), *Angelica archangelica* L. (roots) (*A. archangelica* roots), *Thymus vulgaris* L. (*T. vulgaris*), *Hyssopus officinalis* L. (*H. officinalis*), *Nepeta cataria* L. (*N. cataria*), *Echinacea purpurea* L. Moench. (*E. purpurea*), *Salvia officinalis* L. (*S. officinalis*) and *Desmodium canadence* L. DC. (*D. canadence*).

Extracts were prepared in ambient temperature (ca. 20°C). 0.5 g of ground raw material of the plant was added to a 20 ml vial and was extracted using a 10 ml of 40% (v/v) EtOH/water mixture. The extraction lasted for 24 hrs in an orbital shaker at 180 rpm.

Analytical procedures and cytotoxicity evaluation

Capillary electrophoresis–contactless conductivity detection. Capillary electrophoresis-contactless conductivity detection (CE-C4D) was performed following the previously published, slightly modified procedure, where analysis was done in acidic media [21]. Separation was carried out using a 0.5 M acetic acid background electrolyte (pH 2.5) at 40°C. Hydrodynamic injection was used (50 mbar × 40 s). Separations were done in the fused silica capillary: inner diameter 50 µm, outer diameter 365 µm, total length 50 cm, effective length 38 cm. Prior to the analysis, the capillary was washed with the 0.1 M NaOH solution and the background electrolyte.

Gas chromatography–mass spectrometry. The samples were injected in a gas chromatography injection port using a micro syringe, 1 µl was injected using a split mode at the split ratio 1:10. The linear velocity of the mobile phase was set to 1.2 ml/min in the column. Temperature gradient conditions were set starting from 60°C and maintained for 3 min, then rising to 150°C at 5°C/min and from 150 to 180°C at 20°C and maintaining the final temperature for 3 min. The injection port and the column interface were set to 240 and 260°C, respectively. The ion source was heated to 260°C. The electron ionization mode was used for mass spectrometry with 70 eV ionization energy. Masses were scanned in the range from 30 to 400 m/z.

Spectrophotometric analysis. Determination of total phenolic content (TPC). TPC was determined following the previously optimized Folin–Ciocalteu method [10, 22]. 1.0 ml of the sodium carbonate (4%, pH 10) solution was mixed with 0.033 ml of the sample and 0.033 ml of the Folin–Ciocalteu reagent (2 N). The reaction lasted for 30 min at 30°C and after the reaction absorbance was measured at 760 nm wavelength. The Rutin standard solution was used for the calibration and the determined amounts were expressed in rutin equivalents (RE mg/ml). The calibration range was 0.01–1.00 mg/ml.

Determination of total flavonoid content (TFC). TFC was determined following the previously optimized procedure [10, 22]. The stock solution was prepared and it consisted of 60.0 ml methanol, 3.0 ml acetic acid (33%), 12.0 ml hexamethylenetetramine (5%), 9.0 ml aluminum chloride (10%) and 60.0 ml bidistilled water. The sample (0.04 ml) was added to a 0.960 ml stock solution. The reaction lasted for 30 min at +2°C. After the reaction absorbance was measured at 407 nm. The calibration range was 0.01–1.00 mg/ml.

Determination of radical scavenging activity (RSA). RSA was determined following the previously optimized procedure [10,22]. The sodium acetate buffer (0.1 M) was prepared (pH 5.5). In 125 ml ACN with 125 ml MeOH 10 mg DPPH was dissolved. The obtained organic mixture was mixed with the acetate buffer (1:1). The absorbance was recorded at 515 nm wavelength and the DPPH reagent was diluted with ACN, so that the absorbance showed 0.6. The sample (0.025 ml) was added to the DPPH reagent (1.0 ml). After 15 min (30°C) the absorbance was measured at 515 nm. The calibration range was 0.01–0.50 mg/ml.

Cytotoxicity evaluation. The cytotoxic concentration (CC_{50}) was determined for each extract on Vero cells using the MTT assay [23]. First, cells were seeded at a concentration of 1×10^4 cells/well in a 96-well plate and grown at 37°C for 1 day. The assay was performed in octuplicate for each extract. After 72 hrs the MTT reagent (10 µl, 5 mg/ml) was added and incubated for 4 hrs at 37°C. Then 100 µl dimethyl sulphoxide (DMSO) was added to each well and the plates were placed on the shaker for 5 min. The absorbance of each well was measured at 620 nm in a microplate reader (Multiskan™ FC Microplate Photometer) and the percentage of cell survival was calculated. Finally, dose-response curves were plotted to enable the calculation of CC_{50} that causes lysis and death of 50% of cells.

Data analysis

Data normalization and conditioning. The data points were normalized following the previously published procedure [17]. The average of the dataset is subtracted from the data point and the difference is divided by the standard deviation of the dataset. Using this technique, the statistical average is transformed to 0 and the standard deviation (σ) is transformed to 1. Such data normalization helps avoiding dimensionality and weight difference of the determined chemical parameters in data analysis.

Separation data segmentation. Electropherograms and gas-chromatograms were segmented following the previously published procedure: (i) the pre-electroosmotic region of the electropherograms was sub-divided into 3 segments including high migration velocity, medium migration velocity and low migration velocity peaks (cations in the pre-electroosmotic region) of the electropherograms; (ii) the chromatograms were sub-divided into 3 segments including high (5–9 min), medium (9–16 min) and low (16–24 min) volatility substances; (iii) the peak areas were summed in the segment; (iv) peak sums of the segments were used for further data analysis.

Hierarchical clusterization. Hierarchical clusterization was performed using the software Rstudio [24]. The dataset was imported into the software and for clusterization the method of Euclidean distance matrix calculation was used.

Classification and regression tree induction. The classification and regression tree (CART) decision tree induction was performed using the software Rstudio with the additional package rpart [25].

Multidimensional scaling (MDS) [26]. MDS was performed using the Rstudio software. For calculating

the differences, the method of Euclidean distance was used. The function of MDS called cmdscale was applied and all dimensions were transported to two-dimension projections.

Method validation. Analytical method validation was done in accordance with the pharmaceutical method validation guidelines (ICHQ2R1) [27]. Calibration was performed using at least 6 different levels of concentration in the specified range. Precision and accuracy (expressed in the relative standard deviation) was evaluated from at least 5 different levels of concentrations performing a triplicate analysis.

RESULTS AND DISCUSSION

Characterization of the extracts

Cytotoxicity of the extracts was determined. It was found that the CC_{50} concentration was in the range between the 0.06 (*A. foeniculum*) to 0.77 (*P. frutescens*) dilution of the initial extract, where the lowest values were considered showing the highest cytotoxicity (low concentration is needed for cytotoxic action) and the highest values were considered showing the lowest cytotoxicity. The mean of the dataset was 0.396 and it was decided to classify plant extracts according to the CC_{50} mean value: the extracts possessing higher CC_{50} than the mean (0.396) were classified as non-cytotoxic and the extracts possessing lower CC_{50} than the average were classified as cytotoxic. Cytotoxic and non-cytotoxic extracts were discretized attributing 0 to the non-cytotoxic class and attributing 1 to the cytotoxic class. Such discretization was used for latter experiments and mathematical calculations.

A chemical analysis of the extracts was performed. Different dimensions of chemical attributes were obtained: 11 dimensions corresponding to the chemical attributes and 1 dimension corresponding to the cytotoxicity class (total 12 dimensions). The results were represented in the Table. 1st, 2nd and 3rd attributes corresponded to capillary electrophoresis peak segments and was in the range of 0.012 to 0.47 pF·s. 4th, 5th and 6th attributes were spectrophotometric investigations: TPC, TFC and RSA correspondingly and ranged between 0.04 to 13.47 RE mg/ml. 7th attribute represented the total peak area of the gas chromatogram and was in the range between $1.1 \cdot 10^7$ to $9.1 \cdot 10^8$ relative area units (RAU). 8th attribute represented the number of peaks in the gas chromatogram. The lowest number (19) of peaks was found in the *P. frutescens* extract and the highest number of peaks (47) was found in *S. montana* extract. 9th, 10th and 11th attributes represented segments of gas chromatograms and ranged between 0 to $3.5 \cdot 10^8$ RAU.

Chemical attribute selection for cytotoxic activity

An additional column of the class (cytotoxic – 1 and non-cytotoxic – 0) was added to the dataset in the Table. Some values between the columns differed by 10 decades therefore normalization was performed and this procedure ranged over all values so that the mean of the column was

Table. **Representation of chemical analysis attributes of different plant extracts**

Plant	1	2	3	4	5	6	7	8	9	10	11
Dimension	pF·s	pF·s	pF·s	RE mg/ml	RE mg/ml	RE mg/ml	RAU*	#	RAU*	RAU*	RAU*
S. montana	0.032	0.014	0.017	4.69	1.15	8.29	2.3E+08	47	7.7E+07	1.1E+08	4.8E+07
Ch. nobile	0.089	0.123	0.125	2.05	0.39	1.95	4.7E+08	39	1.2E+08	3.5E+08	0.0E+00
P. frutescens	0.047	0.077	0.012	2.88	0.95	3.11	8.6E+07	19	1.5E+07	6.4E+07	7.5E+06
A. foeniculum	0.066	0.021	0.114	2.93	2.04	3.15	2.2E+08	21	7.3E+07	1.4E+08	4.7E+06
O. vulgare	0.032	0.007	0.011	7.31	2.14	11.98	3.1E+08	42	1.1E+08	1.6E+08	4.2E+07
M. piperita	0.060	0.069	0.037	5.48	2.23	8.72	9.1E+08	40	2.1E+08	6.4E+08	5.7E+07
G. macrorrhizum	0.066	0.155	0.049	7.88	2.49	13.47	4.6E+07	26	1.5E+07	2.9E+07	2.7E+06
M. officinalis	0.056	0.015	0.034	8.02	0.79	13.21	1.0E+08	32	7.1E+07	2.9E+07	3.6E+06
A. archangelica (aerial part)	0.107	0.262	0.049	3.33	0.61	3.39	1.6E+08	39	6.9E+07	7.7E+07	1.4E+07
A. archangelica root	0.472	0.274	0.027	0.75	0.04	0.33	4.1E+08	33	3.0E+08	5.4E+07	5.8E+07
T. vulgaris	0.031	0.003	0.009	5.50	1.51	9.18	2.9E+08	30	1.1E+08	1.3E+08	4.9E+07
H. officinalis	0.050	0.082	0.047	4.42	0.20	7.31	4.6E+08	36	1.6E+08	2.7E+08	3.2E+07
N. cataria	0.075	0.046	0.137	1.53	0.43	1.25	2.2E+08	28	4.5E+07	6.7E+07	1.1E+08
E. purpurea	0.107	0.121	0.116	5.56	0.55	5.37	2.4E+07	21	1.1E+07	1.3E+07	6.1E+05
S. officinalis	0.042	0.028	0.032	5.66	1.79	7.65	5.9E+08	30	2.6E+08	3.2E+08	1.8E+07

* RAU is relative area units.

attributed to 0 and the standard deviation of the column was attributed to 1. For attribute selection, what factors are important, the correlation matrix was calculated using all 12 normalized dimensions (Fig. 1).

As shown in Fig. 1, it was observed that medium and low electrophoretic mobility cations have a tendency to be more abundant in cytotoxic extracts than other compounds. This is interpreted by the fact that the correlation coefficient between the medium electrophoretic mobility cations and the class was 0.46. Additionally, the correlation coefficient between the low electrophoretic migration cations and the class was 0.45. In the previous work, the medium and low electrophoretic mobility cations had a tendency to be less abundant in the extracts that possessed antiviral activity [17]. These compounds can be associated with amino group containing substances, or other positively chargeable organic cations: (i) free amino acids, (ii) biogenic amines, (iii) anthocyanins.

The correlation matrix showed that TPC and RSA attributes have a tendency to be less abundant in cytotoxic extracts. Even though there is a weak tendency, the extracts that possess a high amount of radical scavenging phenolic compounds have a lower probability to be toxic. What is more important, TPC and RSA provided a very high intercorrelation between attributes (0.97), therefore it is stated that both attributes possess the very same information and only one should be used for latter investigations. In the previous work, where antiviral attributes were clarified, it was observed that TPC and RSA abundancy values were significantly higher in antiviral extracts [17].

Interestingly, the attribute values that were obtained from the gas-chromatographic analysis of volatile compounds did not show a high level of correlation. It can be explained by the following presumptions: (i) the volatile compounds in the selected plant extracts do not possess cytotoxic activity, (ii) the cytotoxic activity of volatile compounds in the investigated plants is complex and needs more sophisticated data analysis means, (iii) the cytotoxic volatile compounds in the investigated plants are at low concentrations, therefore the correlation matrix does not show any dependencies and this investigation needs more sophisticated data analysis means.

For further analysis, 2nd, 3rd and 4th (medium and low electrophoretic mobility cations and TPC) attributes were selected and used.

Data mining and chemometrics

In order to confirm the findings described in the *Chemical attribute selection for cytotoxic activity* section, additional data mining techniques were performed: (i) hierarchical clusterization and (ii) multidimensional scaling. The hierarchical clusterization was performed using selected attributes and a cluster dendrogram was generated (Fig. 2). In the dendrogram it is visible that 2 clusters are formed: (i) *non-toxic* and (ii) *toxic* extracts. Three outliers were identified: (i) 2 were found in the *non-toxic* cluster (*M. piperita* and *S. officinalis*) and (ii) 1 was found in the *toxic* cluster (*E. purpurea*).

In the non-toxic cluster it is visible that *O. vulgare* and *M. officinalis* slightly differ from the rest data-points. In the toxic cluster it is visible that 3 sub-clusters are formed: (i) *A. archangelica* (roots and aerial part), (ii) *Ch. nobile*, *A. foeniculum* and *N. cataria*, (iii) *G. macrorrhizum* and *D. canadence*. Even though, there are 2 outliers in the *non-toxic* cluster, it is fully composed of *Lamiaceae* family plants therefore it can be presumed that the clusterization is also sensitive to differences of chemical attributes in different family plants.

1	2	3	4	5	6	7	8	9	10	11	12	
1.00	0.71	−0.02	−0.51	−0.46	−0.48	0.08	−0.01	0.52	−0.19	0.19	0.32	1
	1.00	0.10	−0.37	−0.41	−0.39	−0.10	−0.03	0.16	−0.20	−0.14	**0.46**	2
		1.00	−0.31	−0.24	−0.38	−0.19	−0.38	−0.33	−0.08	−0.08	**0.45**	3
			1.00	0.60	0.97	−0.18	−0.04	−0.27	−0.06	−0.37	**−0.31**	4
				1.00	0.61	0.12	−0.08	−0.09	0.25	−0.15	0.15	5
					1.00	−0.13	0.06	−0.21	−0.03	−0.26	**−0.30**	6
0.46	Moderate positive correlation					1.00	0.52	0.81	0.93	0.38	0.27	7
	indicates cytotoxic effect						1.00	0.42	0.46	0.37	−0.12	8
								1.00	0.57	0.33	0.27	9
−0.31	Weak negative correlation								1.00	0.18	0.23	10
	The extracts possessing these compounds									1.00	0.05	11
	are expected to be less cytotoxic										1.00	12

Fig. 1. Correlation matrix of the chemical analysis attributes and cytotoxicity class. Attributes: (1) high, (2) medium, (3) low electrophoretic mobility cations, (4) TPC, (5) TFC, (6) RSA, (7) volatile compound peak area, (8) volatile peak count, (9) volatile peak area in the segment 4–9 min, (10) volatile peak area in the segment 9–16 min, (11) volatile peak area in the segment 16–24 min, (12) cytotoxicity class

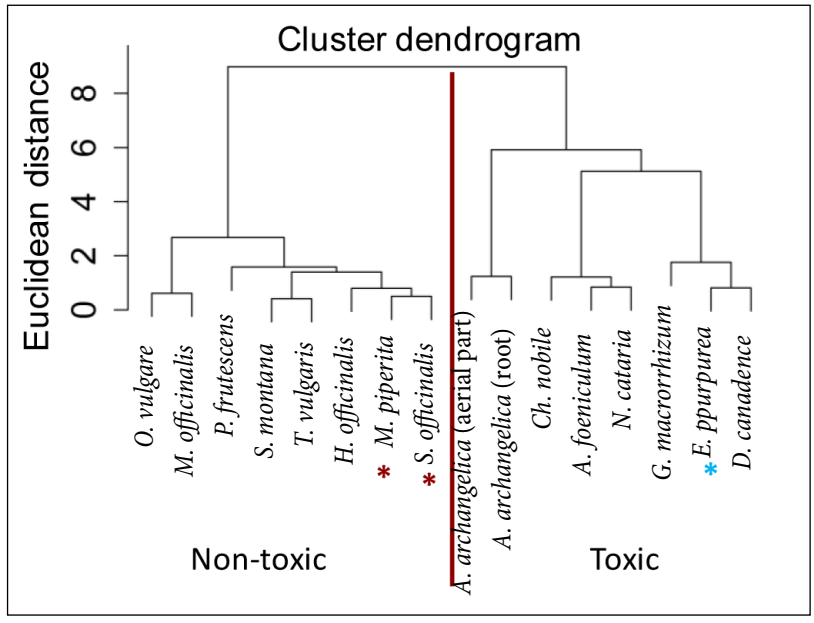

Fig. 2. The cluster dendrogram of medicinal plant extracts according to the selected attributes. * Indication of the outliers: (dark red) *cytotoxic,* (light-blue) *non-cytotoxic*

In order to confirm the findings described in the dendrogram, multidimensional scaling calculations were performed using the selected attributes (Fig. 3). This technique is capable of reducing multiple components to the selected number of dimensions, therefore it is more understandable for the researchers [28]. In this research, the MDS technique provided two projections that were called Coordinate 1 and Coordinate 2. These values were dimensionless, but provided the key information of the clusters in the dataset (Fig. 3).

The same tendency as one observed in hierarchical clusterization dendrogram is visible in the MDS plot. The same outliers were observed: 6 – *M. piperita*, 14 – *E. purpurea*, 15 – *S. officinalis* and one additional – 7 – *G. macrorrhizum*. This is probably due to the fact that the representation of multidimensional data in only two dimensions is usually less accurate than the representation in a multidimensional format. On the other hand, the multidimensional format is less intuitive and often tendencies become invisible for the researchers.

The CART decision tree was generated using the selected attributes with the aim to inducing the rules that determine cytotoxic activity in the extracts (Fig. 4A). The decision tree provided 2 clear rules. If (i) medium electrophoretic mobility cations have a lower normalized value than –0.83, the extract is *non-cytoxic*. If medium

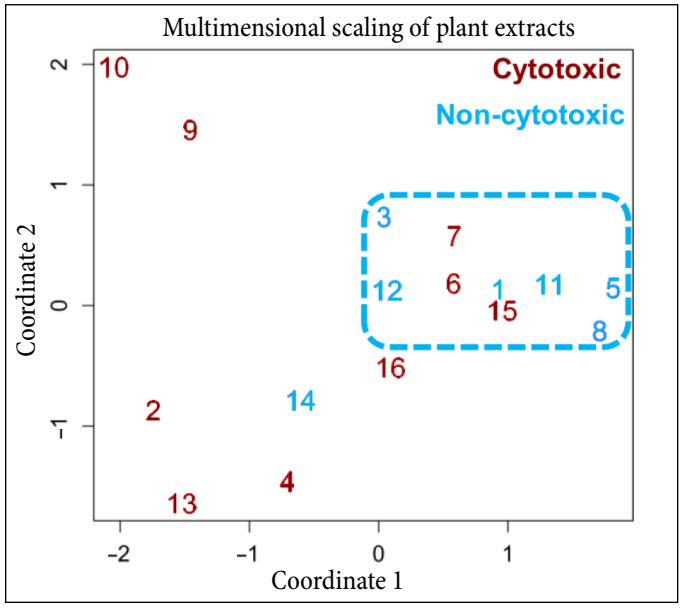

Fig. 3. Multidimensional scaling plot of the selected attributes of the plant extracts: (red) cytotoxic extracts and (blue) non-cytotoxic extracts. Extracts: *S. montana* (1), *Ch. nobile* (2), *P. frutescens* (3), *A. foeniculum* (4), *Origanum vulgare* (5), *M. piperita* (6), *G. macrorrhizum* (7), *M. officinalis* (8), *A. archangelica* aerial part (9), *A. archangelica* root (10), *T. vulgaris* (11), *H. officinalis* (12), *N. cataria* (13), *E. purpurea* (14), *S. officinalis* (15), *D. canadence* (16)

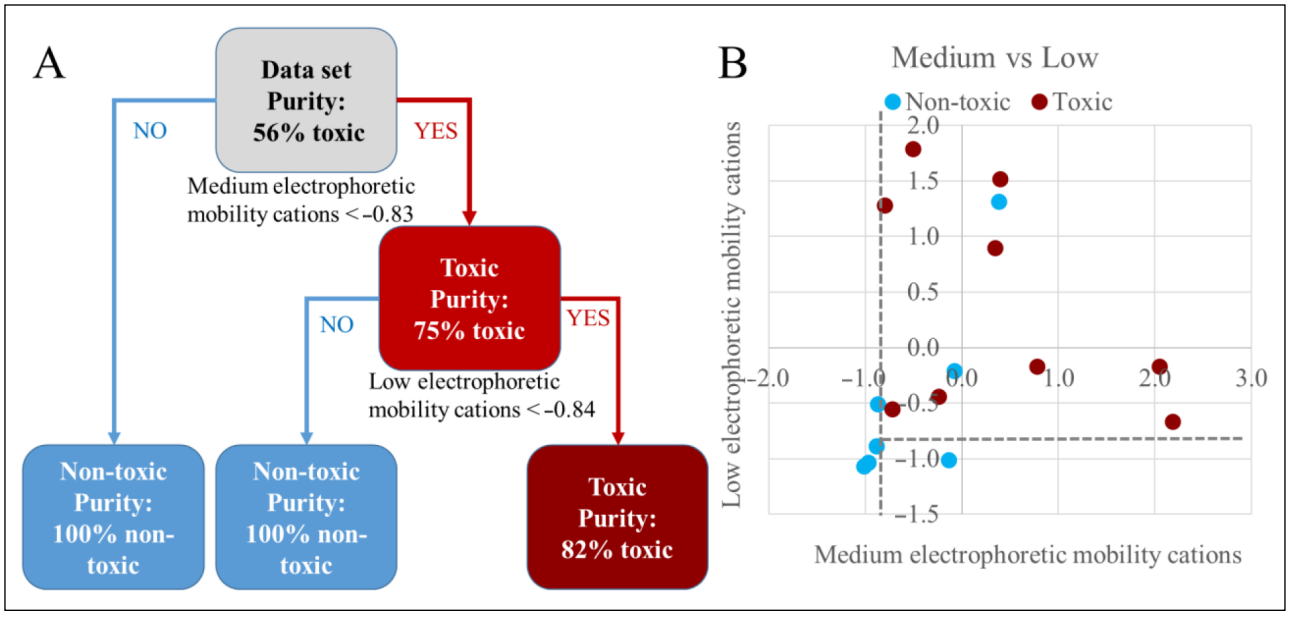

Fig. 4. Representation of the generated CART decision tree. (A) CART decision tree, (B) separation surface of the generated CART decision tree. A grey dashed line shows the rule generating surface

electrophoretic mobility cations have a higher normalized value than –0.83, the extract is *cytotoxic* with a probability of 75%. If (ii) the extract in the second level has the normalized value of low electrophoretic mobility cations lower than –0.84, the extracts are *non-cytotoxic*. If low electrophoretic mobility cations have a higher normalized value than –0.84, the extract is *cytotoxic* with a probability of 82%. The normalized value of –0.83 of medium electrophoretic mobility cations corresponds to the 0.018 pF·s absolute value of the peak area in the segment of medium electrophoretic mobility cations. And the normalized value of –0.84 of low electrophoretic mobility cations corresponds to the 0.019 pF·s absolute value of the peak area in the segment of low electrophoretic mobility cations.

In Fig. 4B the rule generating surface is represented (a grey dashed line). It is visible that low concentrations of medium and low electrophoretic mobility cations are expected in the *non-cytotoxic* extracts.

In the previous work it was clarified that antiviral extracts possessed phenolic compounds with radical scavenging activity and medium and low electrophoretic mobility cations were found to be at low concentrations. This work shows a clear tendency that the cytotoxic extracts have abundant concentrations of medium and low electrophoretic mobility cations. Such observations should be taken into account designing new antiviral phyto-pharmaceutical products and natural virus prevention measures.

CONCLUSIONS

This study shows that: (i) the medium electrophoretic mobility cations are related to the cytotoxic activity of the plant extracts, (ii) the low electrophoretic mobility cations are related to the cytotoxic activity of the plant extracts, (iii) the phenolic compounds possessing radical scavenging activity are expected to be at low concentrations in cytotoxic extracts.

ACKNOWLEDGEMENTS

The research was granted by the Research Council of Lithuania, Project No. MIP-065/2015. The Milton Roy Spectronic 1201 Spectrophotometer was a donation of Alexander von Humboldt Foundation. The capillary electrophoresis system HP3DCE was kindly donated by Dr. Gerard Rozing and Dr. Hans-Peter Schiefer (Agilent Technologies, Germany).

Received 20 March 2018
Accepted 5 April 2018

References

1. Z. Hussain, H. E. Thu, A. N. Shuid, P. Kesharwani, S. Khan, F. Hussain, *Biomed. Pharmacother.*, **93**, 596 (2017).
2. S. Chandra, D. S. Rawat, *Integr. Med. Res.*, **4**, 123 (2015).
3. S. A. El-Toumy, J. Y. Salib, W. A. El-Kashak, C. Marty, G. Bedoux, N. Bourgougnon, *Food Sci. Hum. Wellness*, **7(1)**, 91 (2018). DOI: 10.1016/J.FSHW.2018.01.001.
4. L. Kristanc, S. Kreft, *Food Chem. Toxicol.*, **92**, 150 (2016).
5. N. Tamilselvan, T. Thirumalai, P. Shyamala, E. David, *J. Acute Dis.*, **3**, 85 (2014).
6. L. Ranjbar, J. P. Foley, M. C. Breadmore, *Anal. Chim. Acta*, **950**, 7 (2017).
7. Y. Jiang, M.-Y. He, W.-J. Zhang, et al., *Chinese Chem. Lett.*, **28**, 1640 (2017).
8. J. Haggarty, K. E. Burgess, *Curr. Opin. Biotechnol.*, **43**, 77 (2017).

9. P. Kubáň, D. Štěrbova, V. Kubáň, *Electrophoresis*, **27**, 1368 (2006).
10. V. Kaškonienė, A. Maruška, I. Akuņeca, et al., *Nat. Prod. Res.*, **30**, 1373 (2016).
11. A. Maruška, R. Ugenskienė, D. Raulinaitytė, et al., *Adv. Med. Sci.*, **62**, 158 (2017).
12. E. Alpaydın, *Introduction to Machine Learning*, 1st edn., Vol. 1107, Cambridge University Press, Cambridge (2014).
13. M. Wesołowski, B. Suchacz, *Anal. Bioanal. Chem.*, **371**, 323 (2001).
14. C. Ruiz-Samblás, J. M. Cadenas, D. A. Pelta, L. Cuadros-Rodríguez, *Anal. Bioanal. Chem.*, **406**, 2591 (2014).
15. G. Kaur, H. Monga, *Int. J. Comput. Sci. Inf. Technol. Secur.*, **2**, 2249 (2012).
16. T. Drevinskas, G. Naujokaitytė, A. Maruška, et al., *Carbohydr. Polym.*, **173**, 269 (2017).
17. T. Drevinskas, R. Mickiene, A. Maruška, et al., *Anal. Methods*, **10**, 1875 (2018). DOI: 10.1039/C8AY00318A.
18. T. Drevinskas, M. Kaljurand, A. Maruška, *Electrophoresis*, **35**, 2401 (2014).
19. T. Drevinskas, A. Maruška, V. Briedis, *Electrophoresis*, **36**, 292 (2015).
20. A. Trivellini, M. Lucchesini, R. Maggini, et al., *Ind. Crops Prod.*, **83**, 241 (2016).
21. T. Drevinskas, V. Bartkuvienė, A. Maruška, *Chemija*, **25**, 206 (2014).
22. V. Kaškoniene, M. Stankevičius, T. Drevinskas, et al., *Phytochemistry*, **115**, 184 (2015).
23. T. Mosmann, *J. Immunol. Methods*, **65**, 55 (1983).
24. *RStudio Team* (2015).
25. T. Therneau, B. Atkinson, B. Ripley, M. B. Ripley, *rPart: Recursive Partitioning and Regression Trees*, R Package Version 4.1–9 (2015).
26. P. J. F. Groenen, I. Borg, *The Past, Present, and Future of Multidimensional Scaling*, Econometric Institute Report EI 2013-07, Econometric Institute, Erasmus School of Economics (ESE), Erasmus University Rotterdam (2013).
27. *ICH Topic Q2 (R1). Validation of Analytical Procedures: Text and Methodology* (2005).
28. I. Borg, P. Groenen, *J. Stat. Softw.*, **31**, 1 (2009).

Tomas Drevinskas, Rūta Mickienė, Audrius Maruška, Mantas Stankevičius, Nicola Tiso, Algirdas Šalomskas, Raimundas Lelešius, Agneta Karpovaitė, Ona Ragažinskienė

AUGALŲ, TURINČIŲ POLIFENOLINIŲ IR LAKIŲ JUNGINIŲ BEI PASIŽYMINČIŲ PRIEŠVIRUSINIU POVEIKIU, CITOTOKSINIAI POŽYMIAI

S a n t r a u k a

Natūralių produktų pritaikymas įvairių ligų gydymui ir prevencijai tampa vis svarbesnis. Kuriami ne tik antibakteriniai natūralūs preparatai, bet tiriamos ir natūralios antivirusinės priemonės. Tyrimuose išskiriamos dvi pagrindinės problemos: (1) būtina naudoti specialius cheminės analizės ir duomenų analizės metodus ir (2) svarbūs ne tik antivirusiniai veiksniai, bet reikia atkreipti dėmesį ir į citotoksinius bei kitus nepageidaujamus poveikius. Darbe aprašytas skirstymo metodų (kapiliarinė elektroforezė ir dujų chromatografija), spektrofotometrinių metodų, duomenų išgavimo / duomenų analizės metodų (hierarchinė klasterizacija, daugiadimensinės skalės keitimas ir klasifikacijos bei regresijos medžių indukcija) pritaikymas aiškinantis, kokie cheminiai procesai augalų ekstraktuose yra atsakingi už citotoksinį poveikį. Tyrimui parinkti augalai, priklausantys: notrelinių, graižažiedžių ir pupinių šeimoms. Citotoksiškiausi buvo *Agastache foeniculum* Pursh. Kuntze. ir *Salvia officinalis* L. ekstraktai. Mažiausiai citotoksiški – *Perilla frutescens* L. ir *Satureja montana* L. ekstraktai. Išaiškinta tendencija, kad citotoksiškai aktyvūs ekstraktai turi reikšmingas vidutiniškai ir lėtai elektroforetiškai migruojančių katijonų koncentracijas.